New Technologies in Alfalfa

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Take Home Messages

New technologies in biotechnology have enabled improvements in alfalfa forage quality, fiber digestibility and an increase in rumen bypass protein not possible through traditional breeding methods.

- Reduction in lignin content through gene knockout can benefit hay growers by delaying harvest seven to ten days without compromising forage digestibility. Delay in harvests can translate into:
  - Fewer harvests
  - Higher yields
  - Longer stand life
- Condensed tannin via insertion of transgenes can slow protein degradation in the rumen and allow more protein bypass and utilization. Benefits of tannin alfalfa can translate into:
  - Decrease in use of protein supplements on dairy
  - Decrease in nitrogen losses to the environment
  - Reduction in bloat when the alfalfa is grazed by ruminants

Introduction

The research presented here is the result of a collaboration of government, industry and private non-profit organizations that formed in 2002 as the Consortium for Alfalfa Improvement. Scientists from the U.S. Dairy Forage

Research Center/ARS-USDA, Forage Genetics International (FGI), Pioneer HiBred and The Samuel Roberts Noble Foundation, Inc., have been focusing their research efforts on improving alfalfa forage quality through improvements in digestibility and protein utilization.

■ Reduced Lignin Alfalfa

As alfalfa plants mature from vegetative to bloom stage, cell wall content (NDF) increases and the digestibility of cell walls (NDFD) decreases (Figure 1). The decrease in NDFD can be primarily attributed to an increase in stem to leaf ratio and consequent increase in lignin content. Lignin is indigestible per se, and cross-links with the cellulose and hemicellulose also decrease the digestibility of these cell wall components.

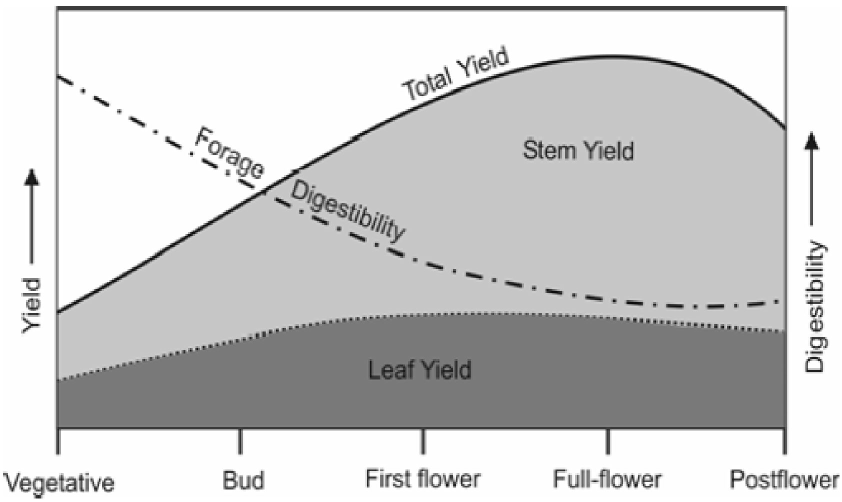

Figure 1. Relative forage yield and quality at different alfalfa growth stages. Source: Balancing Yield, Quality and Persistence. Steve Orloff and Dan Putnam 2004 Proceedings CA Alfalfa Symposium

There are several steps in the process of lignin synthesis in alfalfa. The lignin biosynthetic pathway involves 12 different enzymes, each required for a specific step in the pathway. Noble Foundation scientists used gene silencing technology to "knock out" specific genes that code for specific pathway enzymes. Scientists from FGI took the lead in evaluating elite transgenic reduced lignin (RL) plants in laboratory, greenhouse and field trials for lignin content, lignin composition, NDFD and agronomic characteristics. Of the several knock outs tested, there was only one specific knockout that resulted in the desired product concept – a decrease in lignin content and increase in

NDFD without affecting key agronomic traits such as forage yield, multiple pest resistance, persistence or lodging. FGI produced hundreds of transgenic plants (i.e. events) designed to down-regulate this specific lignin pathway gene, and identified a single event that best captured the desired RL product concept. In addition to meeting all of the necessary agronomic requirements the single event also met the strict metrics required for regulatory approval. From a single transformed plant carrying this elite RL event, FGI breeders developed RL populations designed for use in regulatory trials and also began a process of integrating the RL trait into multiple proprietary breeding lines.

During RL trait development FGI scientists demonstrated that plants containing the commercial RL event (RL alfalfa) had a 15-20% decrease in lignin content and a 10-15% increase in NDFD and Relative Forage Quality (RFQ) compared to related lines without the RL event. They also learned that the rate of change in quality with advancing maturity in RL alfalfa was significantly different than for conventional alfalfa. This difference allows more flexibility in harvest management with a broader harvest window for the production of high quality alfalfa hay/haylage. In various tests designed to better understand this phenomenon, the FGI trait development team learned that RL alfalfa with harvest delayed by seven days had about the same NDFD as conventional alfalfa harvested a week earlier. This was substantiated in cutting management trials comparing RL and conventional alfalfa harvested at 28 day (~late bud) vs. 35 day (~10% bloom) cutting intervals (Figure 2).

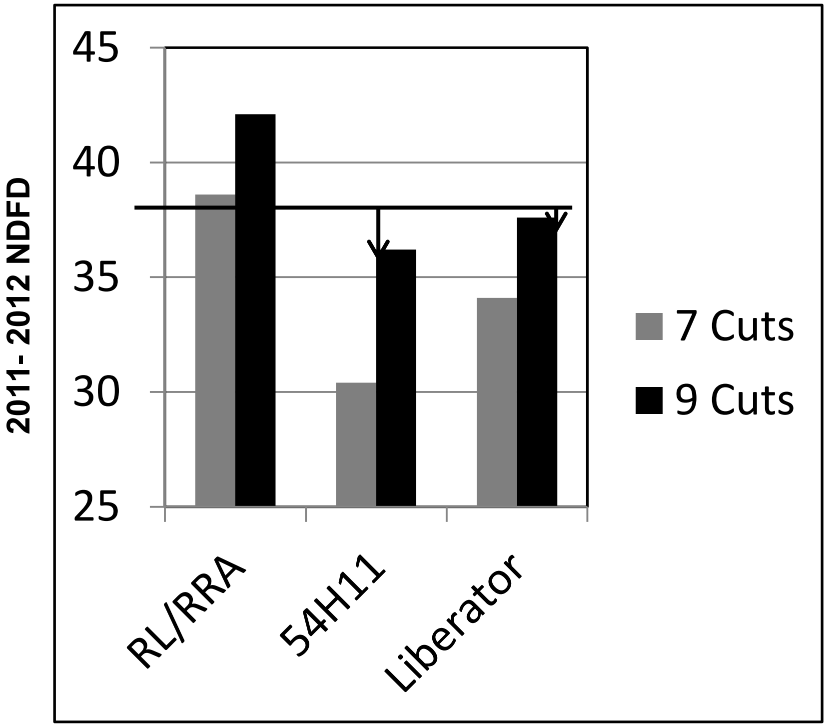

Figure 2. NDFD in RL Alfalfa vs. commercial checks in 3 vs 4 cut management West Salem, WI (established 2010, harvested in 2011 and 2012)

Cutting management trials using conventional alfalfa varieties have demonstrated that early (bud stage) and frequent harvest is often required for the production of high quality hay suitable for feeding to high producing dairy cows but forage yield and stand persistence increase when harvest is delayed until the 10% bloom stage (Marble, 1974; Undersander et al., 2011). For example, trials conducted at the University of Wisconsin have shown a 15-20% forage yield advantage for a three cut vs. four cut management system over a four year rotation (Undersander, personal communication). However forage quality of conventional alfalfa in the three cut treatment was significantly lower than in the four cut treatment. This "forage yield vs. forage quality tradeoff" defines the dilemma for most alfalfa forage producers in designing their cutting management strategy. The potential for delayed harvest of RL alfalfa without sacrificing forage quality provides a potential "high yield/high quality" solution to this historic dilemma and a potential for fewer cuts/season and lower harvest costs.

In preliminary trials of experimental varieties of RL alfalfa, these varieties are showing a 12-15% increase in NDFD and RFQ compared with conventional commercial checks harvested at the same time. This can translate into a ~20 point RFQ advantage and a $15-20T price premium based on current Midwest hay pricing standards.

In October 2014, the CFIA (Canadian Food Inspection Agency) deregulated the reduced lignin trait in Canada. On 10 November, 2014 the United States Department of Agriculture (USDA) deregulated reduced lignin alfalfa in the U.S. In the coming years the reduced lignin trait will be marketed as HarvXtra™ alfalfa.

Product concept for HarvXtra™ alfalfa

- ≥12% increase in RFQ compared to conventional varieties harvested at the same time; or
- ≥ 7 day delay in harvest with the same or better RFQ as a conventional commercial check harvested without the delay

Furthermore we expect that forage yield potential per se, persistence, multiple pest resistance, and lodging tolerance will be similar to conventional varieties harvested on the same cutting schedule.

■ Moving Forward

Dairy Feeding Trials

In 2014 twenty-six DM/tons of HarvXtra™ was produced for feeding studies to be conducted in 2015. Studies are being designed to answer dairy producers' questions:

- How much RL alfalfa should I feed vs. what I feed today of "conventional" alfalfa?
- How does RL alfalfa perform on high corn silage diets?
- Do I need to feed straw with RL alfalfa?
- Can I (should I) feed higher forage levels with RL alfalfa?
- Does RL alfalfa perform differently when ensiled?
- What happens to the feeding value of RL alfalfa when cutting is delayed?
- Do I need to modify starch feeding levels with RL alfalfa?
- Does RL Alfalfa work well in alfalfa grass mix stands?

These tests will be conducted by Calibrate Technologies, US Dairy Forage Research Center and various animal nutritionists at public universities across the U.S.

Forage Testing

Since 2011 forage nurseries and yield trials have been conducted by Forage Genetics, Noble Foundation, USDFRC and University Scientists in PA, WI, IA, KS, CA, ID and WA to select adapted genotypes as parents for future varieties and to evaluate potential varieties for forage quality, yield and persistence. With deregulation of HarvXtra in 2014 the number of locations and cooperators will be expanded to encompass environments representing the entire U.S. hay industry. Tests are also underway in Canada, Mexico and Argentina for submission to their respective regulatory agencies.

HarvXtra™ Release

There will be a limited commercial introduction of HarvXtra™ in 2016 allowing growers the opportunity to realize the value and benefit of the technology. Following deregulation in key export markets HarvXtra™ will be sold to a larger portion of U.S. alfalfa producers.

■ Tannin Alfalfa

Condensed tannins (CT) are a class of phenolic compounds found in many plants. Tannins bind with proteins and slow the rate of protein degradation in the rumen. Reductions in rumen protein breakdown could benefit the dairy industry by reducing:

- The need for supplemental proteins (often the most costly portion of dairy feed)
- Nitrogen and methane emissions attributed to rapid protein breakdown in the rumen and reticulum
- Bloat when alfalfa is grazed (Getachew, 2006)

By elevating the amount of CT in alfalfa it should be possible to increase the amount of protein entering the hindgut of cows, translating into increased milk production and weight gain. Tannin containing forages such as birdsfoot trefoil (*Lotus corniculatus)* and sanfoin (*Onobrychis viciifolia)* have more bypass protein and are non-bloating when grazed by ruminants. Alfalfa produces CT but only in the seedcoat. Various biotech strategies are being explored for production of CT in leaves and stems of alfalfa.

Through overexpression of a key transcription factor, TaMYB14, isolated from rabbit's foot clover (*Trifolium arvense*) scientists from AgResearch in New Zealand and FGI have successfully developed alfalfa plants with expression of CT in the leaves (0.9-1.6%) that are comparable to birdsfoot trefoil checks (1.0%) (Hancock 2014).

The U.S. Dairy Forage Research Center estimates that tannin alfalfa could decrease protein feed supplement costs for dairy by $300 million U.S. and significantly decrease nitrogen losses to the environment (Zeller and Grabber, 2014). In addition, University of California, Davis scientists are exploring strategies for production of hydrolysable tannins in alfalfa that have similar positive attributes of CT.

■ Conclusion

U.S. alfalfa hay production acres have been in gradual decline for the last decade. Biotechnology (applied genomics and genetic engineering) and the potential these tools offer to alfalfa improvement are critical for keeping the crop competitive with alternative genetically engineered crops. Genetic engineering offers potential breakthroughs in improving the efficiency of alfalfa forage production, and improving forage quality in ways not possible with traditional plant breeding methods.

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